

HARPIN FROM *ERWINIA AMYLOVORA* INDUCES PLANT RESISTANCE

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Plants have evolved a complex array of biochemical pathways that enable them to recognize and respond to signals from the environment. A common form of plant resistance is the restriction of pathogen proliferation to a small zone surrounding the site of infection. Typically this restriction is accompanied by localized necrosis. In addition to local defense response, plants also respond to infection by activating defenses in uninfected parts of the plant, which result in resistance of the plant to secondary infection (Dean and Kuc, 1985). Collectively, this phenomenon of induced resistance is called systemic acquired resistance (SAR). SAR reduces the severity of disease caused by all classes of pathogens and it can persist for several weeks or longer. SAR can be induced by abiotic agents, such as salicylic acid as well as biotic agents, such as virulent and avirulent pathogens (Dean and Kuc, 1985; Malamy *et al.*, 1990). Salicylic acid is believed to play a signal function in the induction of SAR since endogenous levels of salicylic acid increase after "immunization" with an incompatible pathogen. However at present, little is known about the signal transduction pathways activated during responses of a plant to attack by a pathogen, although this knowledge is central to understanding disease susceptibility and resistance.

Erwinia amylovora is an often devastating plant pathogenic bacterium that causes the fire blight disease of pear, apple and many other rosaceous plants. In non-host plants, *E. amylovora* elicits the hypersensitive response (HR), which is characterized by a rapid, localized death of tissues infiltrated with high concentrations of bacterial cells ($>10^7$ cfu/ml) (Klement, 1982). *hrp* genes are essential for *E. amylovora* to cause disease in host plants and to elicit the HR in non-host plants (Beer *et al.*, 1991). Harpin is a heat-stable, glycine-rich, secreted protein with molecular mass of 37 kD. It is encoded by *hrpN* of *E. amylovora* (Wei *et al.*, 1992). When infiltrated into intercellular spaces, harpin elicits the HR in many plants including tobacco, pepper, sunflower, tomato cabbage, arabidopsis, cucumber, geranium, watermelon and lettuce.

The HR is believed to be associated with plant defense against pathogens. Hence, we reasoned that harpin-induced HR may induce plant resistance. We tested harpin-induced resistance in more than seven different plants against eight diseases caused by fungi, bacteria and viruses. All tested plants showed some resistance. Here we report evidence of harpin-induced resistance to three diseases, southern bacterial wilt of tomato, tobacco mosaic virus and Gliocladium leaf spot of cucumber.

Harpin-induced resistance in tomato against southern bacterial wilt caused by *Pseudomonas solanacearum*.

100 μ l of a cell suspension of ca. 10^8 cfu/ml of *Escherichia coli* DH5 α (pCPP430) or 100 μ l of a 200 μ g/ml crude harpin preparations were infiltrated into portions of the two lower true leaves of two-week-old tomato seedlings grown in 8 x 15 cm flats in the greenhouse. Twenty plants were used for each treatment. Necrosis was evident 24 hours after infiltration of harpin or *E. coli* DH5 α (pCPP430), which produces and secretes

harpin. Four days after the tomato seedlings had been treated with harpin or bacteria, they were inoculated with *P. solanacearum* K60 (10^7 cfu/ml) by root dipping for three minutes. The inoculated plants were replanted into the same flats and left in a greenhouse. None of the 20 harpin-infiltrated plants showed any symptoms one week after inoculation with *P. solanacearum* K60. However, seven of the 20 buffer-infiltrated plants were stunted. After two weeks, 11 buffer-infiltrated plants showed severe wilting and five were stunted, characteristics of the southern bacterial wilt disease. In comparison, only two harpin-treated plants appeared wilted and three plants were stunted. Similar induced resistance was observed following infiltration of living bacteria *E. coli*/DH5 α (pCPP430), but not by *E. coli* DH5 α (pCPP430), which is a harpin-deficient mutant created by transposon Tn5tac insertion into the *hrpN* gene. These results indicate that harpin, which is produced and secreted by *hrp* gene cluster of *E. amylovora*, is responsible for the induced-resistance realized.

Harpin-induced resistance in tobacco to tobacco mosaic virus (TMV)

One panel of a lower leaf of four-week-old tobacco seedlings (*Nicotiana tabacum* L. "Xanthi" with *N* gene) was infiltrated with 100 μ l of a 200 μ g/ml crude harpin preparation in 5 mM phosphate buffer. Three days later, the plants were challenged with TMV. Fifty μ l of a suspension of TMV (5 μ g/ml) was rubbed on one upper leaf with 400-mesh carborundum. Six plants were used for each treatment. Necrotic lesions appeared on inoculated leaves of both harpin- and buffer-treated plants 4 days after inoculation. The average number of necrotic lesions from the six harpin-treated plants was 21, which was significantly less than the 67 lesion average that developed on six buffer-treated plants. More importantly, the size of the lesions on buffer-treated plants was larger than those on the harpin-treated plants. Actually, it was difficult to distinguish individual lesions on the buffer-treated plants by day 10, because several necrotic lesions had merged.

Harpin-induced resistance against *Gliocladium* leaf spot of cucumber

Harpin or a cell suspension of *E. coli* DH5 α (pCPP430) was infiltrated into first two true leaves of two-week-old cucumber seedlings. Six plants were infiltrated for each treatment. Four days after infiltration of harpin, a *Gliocladium cucurbitae* spore suspension (10^6 spores/ml) was sprayed onto the whole plants. The inoculated plants were incubated in a moisture chamber. Ten days after the inoculation, typical leaf spots appeared. A mean of six lesions was present on the lowest leaves of six harpin-treated plants, but 32 lesions formed on the same leaves of the six buffer-treated plants. On the third lowest leaves, the difference in disease severity was even greater; there were virtually no lesions on harpin-treated plants, however, more than 30 lesions were found on the buffer-treated plants. Later, most of the diseased leaves on buffer-treated plants wilted and died.

The examples outlined above show that harpin is able to induce resistance in different plants against bacterial, viral and fungal pathogens. Although mechanisms of harpin-induced resistance are unknown, some of our preliminary experiments have shown that harpin may act as an elicitor of salicylic acid induction, which is believed to be involved in SAR (Malamy *et al.*, 1990). Unlike some host-specific elicitors (Keen *et al.*, 1990), harpin is able to elicit the HR on a broad range of plants. Thus, we expect that harpin-induced resistance can be achieved in many plants either by manipulation of harpin exogenously or by harpin-mediated transgenic plants.

Our studies of harpin-induced resistance are just beginning and we need to learn more to understand the exciting features of this phenomenon. For example, what is the minimal amount of harpin needed to induce plant resistance and how long does the resistance persist, and what mechanisms are involved in harpin-induced resistance? We expect that harpin as a novel molecule will play an important role in dissecting the signal transduction pathways of induced-resistance in plants, and perhaps also in practical disease control.

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